Abstract No. gavi674

In Situ Cryo-Protection and Anomalous Scattering Atom Derivatization of Protein Crystals: An Application for High Through-put Screening and Data Collection

J.A. Gavira (UAH) and J.D. Ng (UAH)

Beamline(s): X4A

Introduction: The incorporation of strong anomalous scattering atoms in protein crystals and selecting optimal cryogenic conditions for x-ray diffraction traditionally requires lengthy co-crystallization or soaking procedures. This usually involves soaking or co-crystallization of the atoms of interest into the protein crystal by vapor diffusion or batch techniques. Thereafter, suitable cryogenic conditions are further searched by soaking the derivatized crystal into a cryogenic preservative that may render the crystal usuable for data collection. To obtain adequate derivatized crystals soaked in an optimal concentration of cryogenic solution is, therefore, very exhaustive and time consuming. Recently we have shown that counter-diffusion techniques allow the slow incorporation of the cryo-buffer during crystal grown inside an x-ray capillary which can be frozen and diffracted without any manipulation [1]. The integrity and quality of the crystals are preserved when crystal handling is avoided [2-4]. These techniques offer a wide range of improvement for protein crystal growth based on diffusive mass transport. In this work the same idea has been apply to incorporate different strong anomalous scattering halides while self screened for optimal concentration of a cryogenic solution to three models protein, lysozyme, thaumatin and insulin. We show the feasibility of screening protein crystals *in situ* for optimal derivatization of Br and I in the presence of a cryogenic solution inside a capillary tube and used for x-ray data collection.

Methods and Materials: The experiments were performed with the counter-diffusion geometry [5]. Each capillary was filled with two layers of solution. The first layer is prepared by mixing the protein solution with agarose (0.5% w/v) both in buffer in a volume ratio 9:1. The second layer includes the precipitant salt, metal halide (bromide/iodine) and the cryogenic preservation solution (glycerol) with a volume ratio of 5:1:5 respectively. Insulin, lysozyme and thaumatin crystals were grown using this technique inside capillary tubes with inner diameters of 0.5 or 0.3 mm. The capillary regions containing the selected crystal were cut in piece of 2-3 cm. In each piece of capillary, one to three crystals can be frozen at the same time and diffracted individually. X-ray data were recorded on line X4A using a FUJI BAS2000 scanner. The data were processed and reduced with the HKL2000 package [6]. MAD data were collected at three different wavelengths for the bromide and only two for the iodine derivatives. A total of 15 data sets (five for each protein), were collected. The anomalous scattering strength and positions of the halides were revealed from the anomalous difference Patterson maps calculated with Xtalview [7].

Results and discussion: Three model proteins have been crystallized, derivatized with Br and I, and cryo-protected in a single step followed by data collection without any direct handling or manipulation of the crystals. Phases can be determined using SAD, SAS or MAD techniques. The *ab initio* structure determination of these three proteins is in progress. The comparison of the accuracy of the determined structures is still in progress too. We show that protein crystals can be screened for optimal halide derivatization and cryogenic conditions followed by data collection and initial phase determination rapidly and efficiently. This approach has direct application for high through-put structure determination by x-ray crystallography.

Acknowledgments: We express our gratitude to Drs Zhi-Jie Liu and B.C. Wang for their valuable aassistance in the final crystallographic analysis for the usage of the ISAS crystallography package. This work was supported by NASA and NSF.

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